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Research article

Integrated single-cell RNA sequencing and multi-database analysis reveal THBS2 promotes high glucose-induced mesangial cell oxidative stress and fibrosis in diabetic nephropathy ☆

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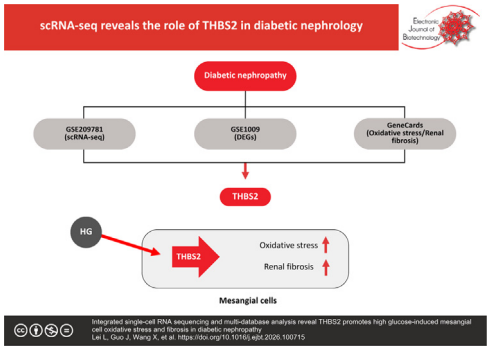
GRAPHICAL ABSTRACT

Integrated single-cell RNA sequencing and multi-database analysis reveal THBS2 promotes high glucose-induced mesangial cell oxidative stress and fibrosis in diabetic nephropathy.



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ABSTRACT

Background: Single-cell RNA sequencing (scRNA-seq) has provided unprecedented resolution for the study of many diseases, including diabetic nephropathy (DN). However, the effective genes regulating oxidative stress and fibrosis in mesangial cells in DN remain to be revealed. In this study, scRNA-seq and multi-database analysis were used to identify thrombospondin 2 (THBS2) as a key gene in regulating DN progression.

Results: The scRNA-seq revealed a total of 6 important cell types in diabetic kidney disease (DKD) from the Gene Expression Omnibus (GEO) database (GSE209781). GO and KEGG analysis showed that the differential genes in mesangial cells of DKD had multi-function and multi-pathway regulation. A total of 4 target genes (ITGB1, THBS2, TIMP1 and COL18A1) were screened by taking the intersection of scRNA-seq (mesangial cells-related genes), GEO database (GSE1009) and GeneCards database (oxidative stress-related genes and renal fibrosis-related genes). THBS2 was overexpressed in high glucose (HG)-induced mesangial cells, and its knockdown inhibited HG-induced mesangial cell oxidative stress and fibrosis.

☆ Audio abstract available in Supplementary material.

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Renal fibrosis
Single-cell RNA sequencing (scRNA-seq)
Thrombospondin 2 (THBS2)

Conclusions: Based on scRNA-seq, multi-database analysis and experimental verification, this study showed that THBS2 might promote HG-induced mesangial cell oxidative stress and fibrosis in DN.
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1. Introduction

Diabetic nephropathy (DN) is the most serious microvascular complication of diabetes, accounting for 40% of end-stage renal disease [1,2]. Intermittent proteinuria is the only clinical manifestation of DN in the early stage, and edema, hypertension and renal insufficiency may occur in the later stage [3,4]. Mesangial cells are essential to maintaining glomerular integrity in the kidney, and its dysfunction can lead to DN progression [5]. Although hyperglucose-induced oxidative stress and fibrosis are known to be central mechanisms of DN progression [6], the key regulators mediating the pathological transformation of mesangial cells are still not fully elucidated. Current clinical interventions can only delay rather than block disease progression [7], which highlights the urgent need for novel molecular targets.

Single-cell RNA sequencing (scRNA-seq) provides unprecedented resolution to study disease by analyzing the gene expression profiles of individual cells and has become a revolutionary tool for disease mechanism research and clinical translation [8,9]. In recent years, scRNA-seq technology has revealed the heterogeneity of renal cells in DN [10,11], but the study about specific molecular events of renal mesangial cells is still insufficient. In this study, scRNA-seq analysis identified key cell types and genes in diabetic kidney disease (DKD) samples. Following, mesangial cells were used as the object, and the key gene thrombospondin 2 (THBS2) was screened by combining the results of scRNA-seq, GEO database and GeneCards database. Previous study showed that THBS2 was upregulated in DN patient kidney samples, and its expression was negatively correlated with renal function indicators [12]. However, whether THBS2 mediates DN progression by regulating oxidative stress and inflammation in mesangial cells under high glucose (HG) is unclear, which constitutes the key scientific question of this study.

2. Materials and methods

2.1. Single-cell transcriptomic data preparation and quality control

The scRNA-seq data were downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo) database (Accession: GSE209781) containing 3 DKD patients and 3 normal (NM) samples (132,013 cell numbers and 27,707 gene numbers). Quality control of scRNA-seq data was performed according to the following criteria: gene feature number, cell number count and mitochondrial sequencing percentage; cells with less than 200 genes and genes covered by less than 3 cells were filtered out; cells with the number of expressed genes less than 200 or more than 6000, the total UMI number less than 20,000 and more than 20% mitochondrial genes were filtered out. The high-quality cells and genes were screened out after quality control (40,438 cell numbers and 26,026 gene numbers). Data normalization was then performed using the "NormalizeData" function in the R package Seurat.

2.2. Cell heterogeneity analysis

Uniform Manifold Approximation and Projection (UMAP) algorithm was used for cluster analysis to reveal the cell heterogeneity in the single-cell dataset. Specifically, the FindNeighbors function in the Seurat package was used to construct the cell-to-cell association network, and the FindClusters function was subsequently used to perform unsupervised clustering analysis. In the clustering parameter Settings, the resolution parameter was selected as 0.2, and the first 15 principal components (PC1-PC15) were used as the input dimensions. The two-dimensional visualization map generated by the RunUMAP function clearly showed the distribution characteristics of the cell population, and finally, 13 cell subsets with unique transcriptional signatures were successfully identified. The "FindAllmarker" function of the R package "Seurat" with thresholds (logfc.threshold = 0.25, min.pct = 0.25, and only.pos = TRUE) was used to identify genes that were specifically highly expressed for each cell cluster.

2.3. Cell type identification

To determine the cell type of the annotated cell cluster, the annotated cell cluster was based on marker genes and TOP highly expressed genes as previously described [13,14]. The cell clusters can be divided into Proximal Convoluted Tubule (PCT), cells of Loop of Henle (LOH), Immune Cells (IMC), Endothelial Cells (ENDO), Mesangial Cells (MES), and Collecting Duct Principal Cells (CD-PC). The cell types after annotation were visualized using UMAP. The expression of Marker genes used for annotation in each cell cluster was also shown in plots.

2.4. Screen for differential cell types and genes

The Wilcoxon rank sum test was used to compare the cell types with significant differences in the proportion between the DKD group and the NM group in the single-cell dataset. $p < 0.05$ was considered as differential cells. The FindMarkers function was used to calculate the differentially expressed genes between DKD samples and NM samples (min.pct = 0.1, logfc.threshold = 0.25, test.use="wilcox"). Volcano plots and heat maps were used to show differentially expressed genes in MES, and the top 10 up-regulated and down-regulated genes were annotated in the plots according to the *p*-value.

2.5. GO and KEGG analysis

The differential genes of MES were imported into Sangerbox for GO/KEGG enrichment analysis. The enrichment method was Benjamini-Hochberg, and the result screening conditions were FDR < 0.1 and *p*-value < 0.05 [15,16]. According to the order of *p*-value, the results of biological process (BP)/cellular components (CC)/molecular function (MF) show the top 10, and KEGG shows the top 20.

2.6. Target gene screening

Genecards database was used to screen genes related to oxidative stress and renal fibrosis. Genes related to oxidative stress were selected for Protein Coding, Relevance score > 1.514761508 (median). Genes related to renal fibrosis were selected for Protein Coding, Relevance score > 2.777264595 (median). GEO database (Accession: GSE1009, including 3 normal samples and 3 DN samples) was analyzed by GEO2R; the screening conditions of differential genes were $p < 0.01$ and FC > 1.2. The differentially expressed genes in MES (1338 genes) were intermixed with GSE1009 (776 genes), oxidative stress-related genes (6470 genes) and renal fibrosis-related genes (3947 genes), and a total of 24 intersection genes were imported into Cytoscape for PPI visualization (font size and color are arranged according to degree value). The top 10 genes were selected by the MCC algorithm in cytohubba plugin, and 4 genes were intermixed by the MCODE plugin. The intersection genes analyzed by Venn diagram were ITGB1, THBS2, TIMP1 and COL18A1.

2.7. Cell culture, treatment and transfection

Human glomerular mesangial cells (CP-H067, Procell, Wuhan, China) were cultured in specific medium (CM-H067, Procell) at 37°C with 5% CO_2. Cells were treated with HG (30 mM D-glucose, Sigma-Aldrich, St. Louis, MO, USA) for 24 h to induce DN cell models, with normal-glucose (NG, 5.5 mM D-glucose) or isotonic mannitol (MNT, 25 mM mannitol + 5 mM glucose) as a control. For transfection, cells were transfected with shRNA against THBS2 (shTHBS2: 5′-CCGGCCCTCCTAAGACAAGGAACATCTCGA GATGTTCCTTGTCTTAGGAGGGTTTTTG-3′) and its negative control (shNC: 5′-CCGGTTCTCCGAACGTGTCACGTAACTCGAGTTACGTGA CACGTTCGGAGAATTTTTG-3′) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA), followed by treatment with HG for 24 h.

2.8. qRT-PCR

TRIzol reagent (Invitrogen) was used to obtain total RNAs from mesangial cells, and cDNA was collected using the cDNA Synthesis Kit (Beyotime, Shanghai, China). Then, qRT-PCR was performed using SYBR Premix Ex Taq™ Kit (TaKaRa, Dalian, China) with specific primers as below: TIMP1, F 5′-CTTCTGCAATTCCGACCTCGT-3′, R 5′-ACGCTGGTATAAGGTGGTCTG-3′; COL18A1, F 5′-CAGTGGACACACT TAGCCCTC-3′, R 5′-GCGGCATTCTCTGGAACTCC-3′; ITGB1, F 5′-CCT ACTTCTGCACGATGTGATG-3′, R 5′-CCTTTGCTACGGTTGGTTACATT-3′; THBS2, F 5′-ATCACACGCATCCGTCTCTG-3′, R 5′-AAACAGC CATTTGGGCATGG-3′; β-actin, F 5′-TCACAATGTGGCCGAGGAC-3′, R 5′-TGGGGTGGCTTTTAGGATGG-3′. Relative TIMP1/COL18A1/ITGB1/THBS2 mRNA expression was determined by the $2^{-\Delta\Delta Ct}$ method.

2.9. Western blot (WB)

Total proteins were isolated by RIPA buffer, separated by SDS-PAGE gel and transferred to PVDF membranes. Membranes were blocked and then incubated with anti-THBS2 (1:1000, ab89805, Abcam, Cambridge, MA, USA), anti-COL-IV (1:1000, 30850-1-AP, Proteintech, Wuhan, China), anti-α-SMA (1:2000, 14395-1-AP, Proteintech), or anti-β-actin (1:50000, 66009-1-Ig, Proteintech). After incubating with secondary antibody (ab205718/ab205719, Abcam), the protein band was detected using ECL reagent (Beyotime).

2.10. CCK8 assay

Mesangial cells were re-seeded into 96-well plates and incubated with CCK8 reagent. At each time point (0, 24, 48 and 72 h), OD value was measured using a microplate reader to assess cell viability.

2.11. Detection of oxidative stress

SOD activity, MDA level and ROS level in mesangial cells were determined using SOD Assay Kit (S0101S, Beyotime), MDA Assay Kit (S0131S, Beyotime) and ROS Assay Kit (S0033S, Beyotime) according to the manufacturer's instructions. SOD and MDA levels were measured under a microplate reader. ROS levels were analyzed by measuring DCFH-DA fluorescence using flow cytometry.

2.12. Statistical analysis

All experiments were performed in triplicate, with each independent experiment set 3 times to generate an average value. Data are evaluated using GraphPad Prism 8.0 software and displayed as mean ± SD. Differences between groups were compared with Student's *t*-test or one-way ANOVA followed by Tukey post-hoc test. $p < 0.05$ was considered significant.

3. Results

3.1. The differential cells and markers were screened by scRNA-seq

Firstly, gene feature number, cell number count and mitochondrial sequencing percentage were used to screen high-quality cells and genes from the scRNA-seq of GEO database (GSE209781), and a total of 40,438 cell numbers and 26,026 gene numbers were obtained (Fig. 1A). Cluster analysis by the UMAP algorithm identified 13 cell subsets with unique transcriptional signatures (Fig. 1B). Cell clusters were then annotated based on marker genes and TOP highly expressed genes in previous studies. The results identified that the cell clusters could be divided into 6 cell types, including PCT, LOH, IMC, ENDO, MES, and CD-PC (Fig. 1C). The number of each cell type in 3 DKD sample and 3 NM samples is shown in Fig. 1D, and the number and proportion of cell types contained in each sample are presented in Fig. 1E. Moreover, the expression of markers in 6 cell types is exhibited in Fig. 1F. Further analysis showed no significant difference in the counts (68, 274, 51 vs 26, 640, 627) and proportion (2.52%, 3.59%, 1.21% vs 0.42%, 6.77%, 18.54%) of MES cells in DKD group and normal group (Fig. S1).

3.2. Differential genes were screened and analyzed by GO/KEGG

According to the pathological characteristics of diabetes, mesangial cells were selected for analysis. The volcano plot and heat map showed the differentially expressed genes in mesangial cells, and the top 10 up-regulated and down-regulated genes were shown according to the *p*-value (Fig. 2A–B). GO and KEGG analysis was used to investigate the functions of differential genes. GO analysis exhibited that the main biological processes were mRNA catabolic process and RNA catabolic process; the main cellular components were extracellular organelle and protein-containing complex; the main molecular function was structural molecule activity and RNA binding (Fig. 3A). KEGG pathway analysis demonstrated that the differential genes participated in multiple important signaling pathways, including Ribosome, Parkinson's disease, oxidative phosphorylation and thermogenesis (Fig. 3B).

3.3. Acquisition of the key target for DN

GeneCards was used to screen the genes related to oxidative stress (6470) and renal fibrosis (3947), and then, the above genes were intermixed with mesangial cell differential genes (1338) and GEO database differential genes (778). A total of 24 intersec-

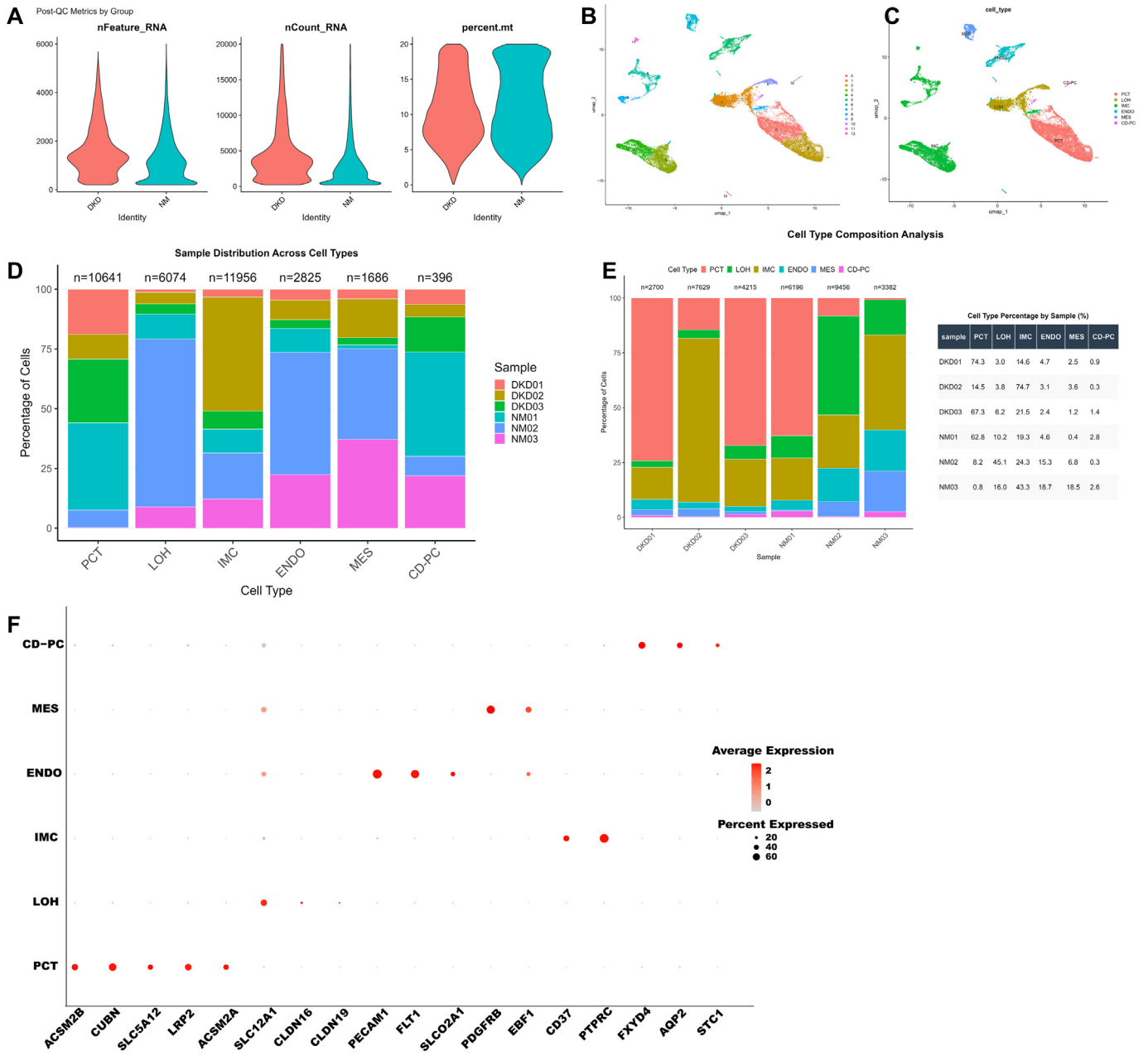

Fig. 1. Scrna-seq screened the differential cells and markers. (A) The screening of high-quality cells and genes based on gene feature number, cell number count and mitochondrial sequencing percentage. (B) UMAP algorithm was used to cluster analysis to identify 13 cell subsets. (C) The cell clusters were annotated based on the marker genes and TOP highly expressed genes. (D) The number of each cell type in 3 DKD sample and 3 NM samples is shown. (E) The number and proportion of cell types in 3 DKD sample and 3 normal samples are presented. (F) The expression of markers in 6 cell types is shown.

tion genes were obtained (Fig. 4A). Following, 24 intersection genes were introduced into Cytoscape software for PPI visualization (Fig. 4B). According to the MCC, the top 10 genes are THBS2, TIMP1, TJP1, UMOD, COL18A1, F2R, HSP90AA1, ITGB1, KNG1 and MYH9. A total of 4 hub genes were screened according to the MCODE score, including THBS2, TIMP1, COL18A1 and ITGB1 (Fig. 4C). The intersection of genes screened by MCC and MCODE is shown in Fig. 4D. To further identify the key target, our study performed preliminary qRT-PCR validation of 4 candidate genes in the HG-induced mesangial cell model. The results showed that all 4 genes were significantly upregulated under HG conditions, with THBS2 being the most obvious (Fig. S2). Combined with its relatively few reports and unknown mechanism research potential in DN, THBS2 was selected for subsequent in-depth functional and mechanistic studies.

3.4. THBS2 knockdown reduced oxidative stress and fibrosis in HG-induced mesangial cells

To confirm the role of THBS2 in DN, the functional experiments were performed. GSE1009 database showed that THBS2 was upregulated in kidney glomeruli from 3 DN patients (Fig. 5A). In cell experiments, NG group, MNT group and HG group were set. CCK8 assay showed that cell viability was significantly higher in the HG group than in the NG and MNT groups, while there was no significant difference between the NG and MNT groups (Fig. S3). Therefore, the mannitol control group was not set up again in the subsequent experiments. Through qRT-PCR, THBS2 was confirmed to be overexpressed in HG-induced mesangial cells (Fig. 5B). Following, shTHBS2 was constructed, and WB results confirmed that transfection of shTHBS2

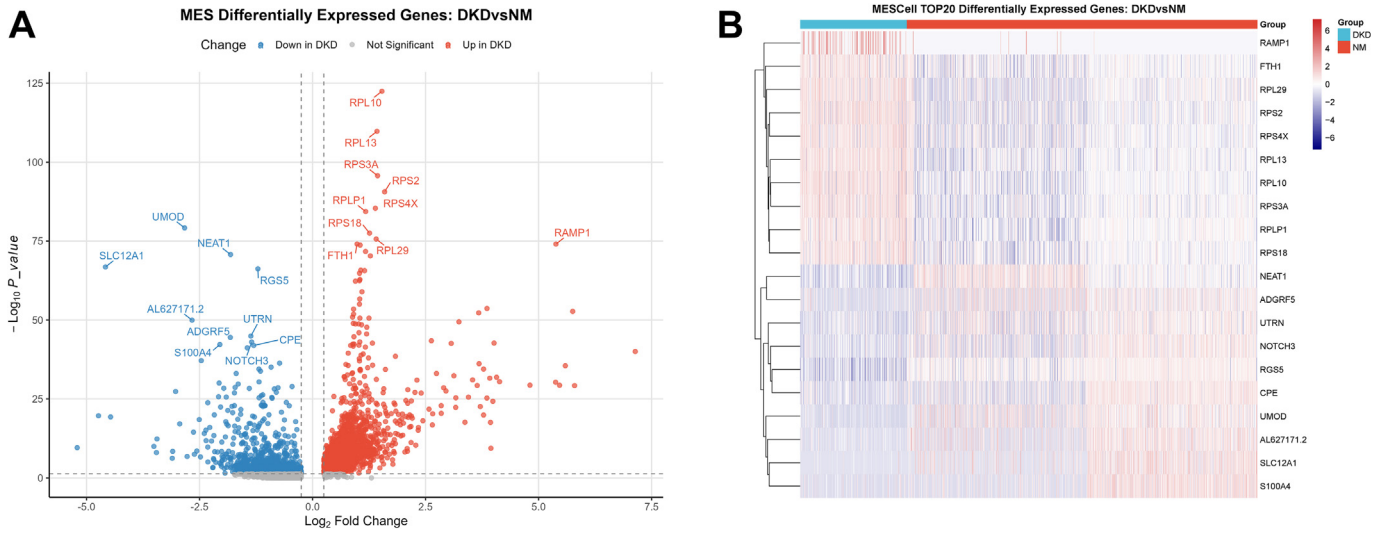

Fig. 2. Screened the differential genes of DN. The differentially expressed genes (top 10 up-regulated or down-regulated) in mesangial cells are shown as volcano plot (A) and heat map (B).

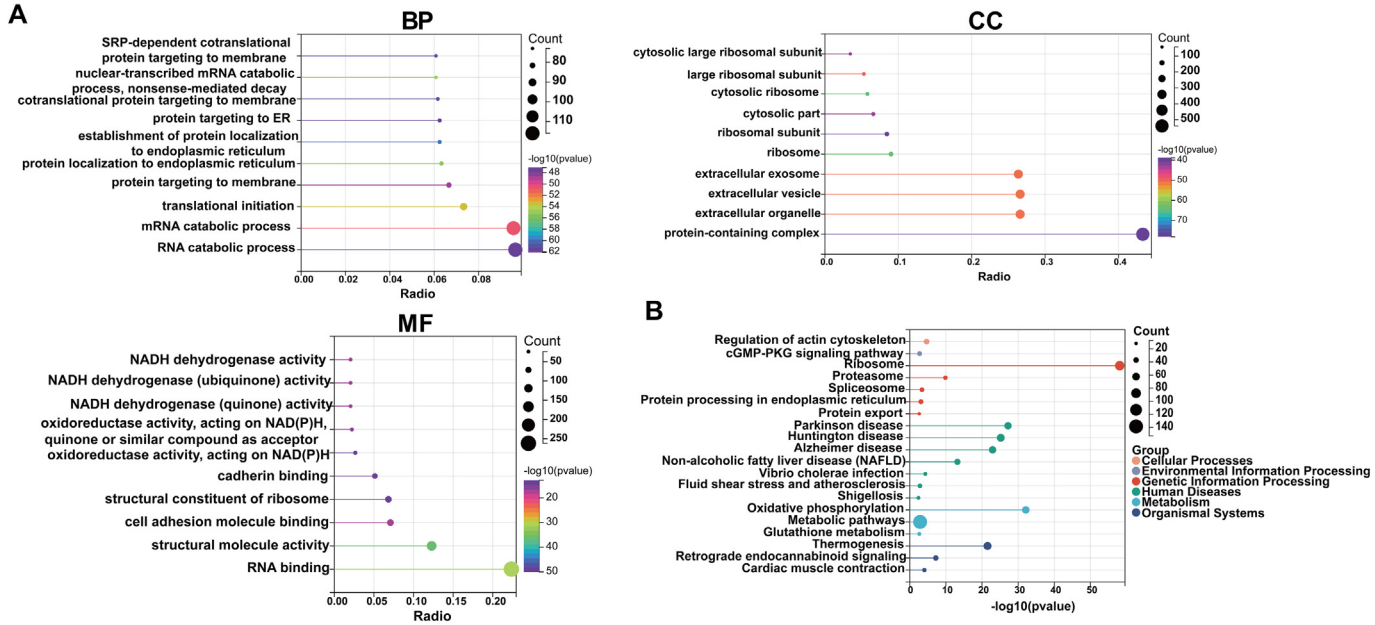

Fig. 3. GO and KEGG analysis. The functions of differential genes in mesangial cells were analyzed by GO analysis (A) and KEGG analysis (B).

was able to significantly reduce THBS2 protein expression in mesangial cells (Fig. S4A–B). Then, mesangial cells were transfected with shTHBS2 and induced by HG. The detection of THBS2 protein level confirmed that shTHBS2 could effectively inhibit THBS2 expression in HG-induced mesangial cells (Fig. 5C–D). HG treatment decreased SOD activity and promoted MDA level in mesangial cells, while THBS2 knockdown reversed these effects (Fig. 5E). Moreover, silencing of THBS2 significantly reduced HG-induced COL-IV and α-SMA protein levels in mesangial cells (Fig. 5F-G). In addition, ROS level was markedly enhanced under HG conditions, which could be reversed by THBS2 knockdown (Fig. 5H). The above data showed that THBS2 might contribute to HG-induced mesangial cells oxidative stress and fibrosis to promote DN progression.

4. Discussion

The progression of DN is complex and involves a variety of cellular and molecular mechanisms, among which mesangial cells play a central role in DN occurrence and development [17]. Mesangial cells are specialized interstitial cells of glomeruli, which may release inflammatory factors, generate ROS, and lead to DNA damage and cell apoptosis under HG conditions, thus promoting DN progression [18,19]. Therefore, elucidation of the key genes affecting mesangial cells can provide insight into the pathogenesis of DN. In this study, scRNA-seq combined with multi-database analysis identified THBS2 as an important gene in mesangial cells, and it promotes HG-induced mesangial cell oxidative stress and fibrosis to accelerate DN progression, which is a novel finding.

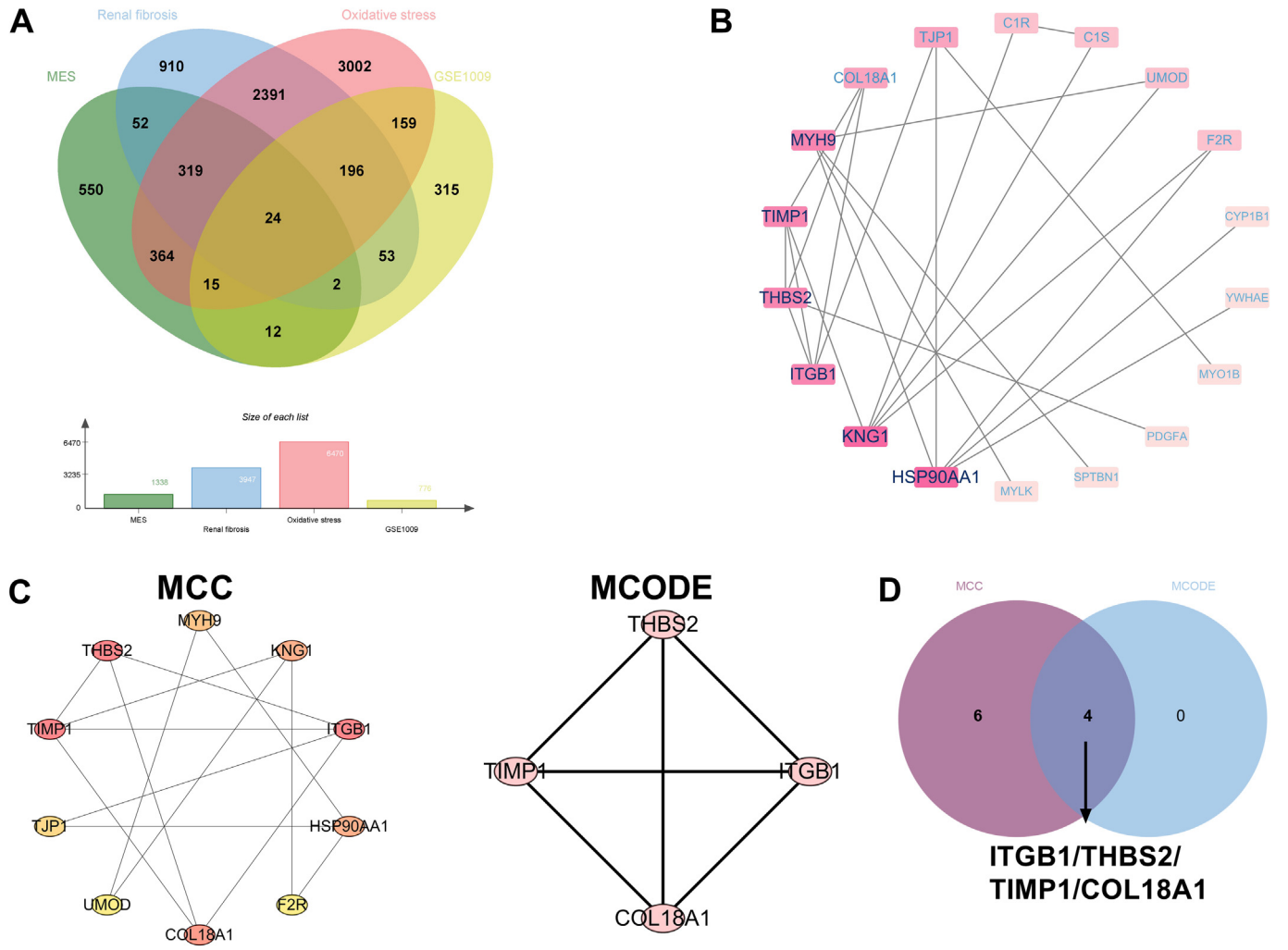

Fig. 4. Key target of DN was screened. (A) Venn diagram was used to screen the 24 intersection genes from oxidative stress-related genes (6470), renal fibrosis-related genes (3947), mesangial cell differential genes (1338) and GEO database differential genes (778). (B) The PPI network of 24 intersection genes was visualized using the Cytoscape software. (C) Top 10 genes were screened according to MCC, and top 4 genes were screened according to MCODE score. (D) Venn diagram was used to obtain the key targets screened by MCC and MCODE.

scRNA-seq technology has revolutionized the prediction of disease-related cells and genes, which can resolve cellular heterogeneity to accurately identify disease-related cell subsets, and can target key genes and pathways by comparing the transcriptome of disease and normal cells [20,21]. Therefore, scRNA-seq is an irreplaceable tool for mechanism research, early diagnosis and precision treatment by analyzing the characteristics of diseases at the cellular and molecular levels [22,23,24,25]. In this study, scRNA-seq was used to analyze the key cells and genes in DKD and NM samples, and 6 important cell types were identified (PCT, LOH, IMC, ENDO, MES, and CD-PC). Combined with previous reports, the expression of marker genes in each cell type was annotated. Given the important role of mesangial cells in DN progression, differential genes in mesangial cells were analyzed in the present study. Further analysis showed that mRNA catabolic process and RNA catabolic process were the main biological processes involved in the differential genes in mesangial cells; extracellular organelle and protein-containing complex were the main cellular components; structural molecule activity and RNA binding were the main molecular functions. Also, this study suggested that Ribosome, Parkinson's disease, oxidative phosphorylation and thermogenesis were important signaling pathways involved in the differential genes in mesangial cells. The above results suggest that the differential genes in mesangial cells may play an important role in DN through multiple pathways.

THBS2 is an extracellular matrix glycoprotein with an elastin domain, which plays a crucial role in regulating many biological processes [26,27,28]. Analysis has shown that the THBS2 gene can increase susceptibility toward DN in type 2 diabetes mellitus patients [29]. Importantly, THBS2 is considered to be a potentially important gene with altered expression in fibrosis [30]. It had been shown that THBS2 silencing repressed TGF-β1-induced lung fibroblast-like cell inflammation, oxidative stress and fibrosis [31]. In a DN-related study, Li et al. [12] confirmed the elevated THBS2 expression in DN samples and reported that THBS2 might be a potential diagnostic marker for DN. Besides, THBS2 could promote DN progression, which suppressed cell proliferation and promoted apoptosis in HG-induced renal tubular cells [32]. The above data confirm the vital role of THBS2 in DN process. By taking the intersection of scRNA-seq, GEO database and Genecards database, THBS2 was identified as an important gene for regulating DN process. Loss-of-function study revealed that THBS2 indeed had increased expression in HG-induced mesangial cells, and its down-regulation restrained HG-induced mesangial cell oxidative stress and fibrosis. These results support the hypothesis that THBS2 promotes HG-induced mesangial cell injury to accelerate DN progres-

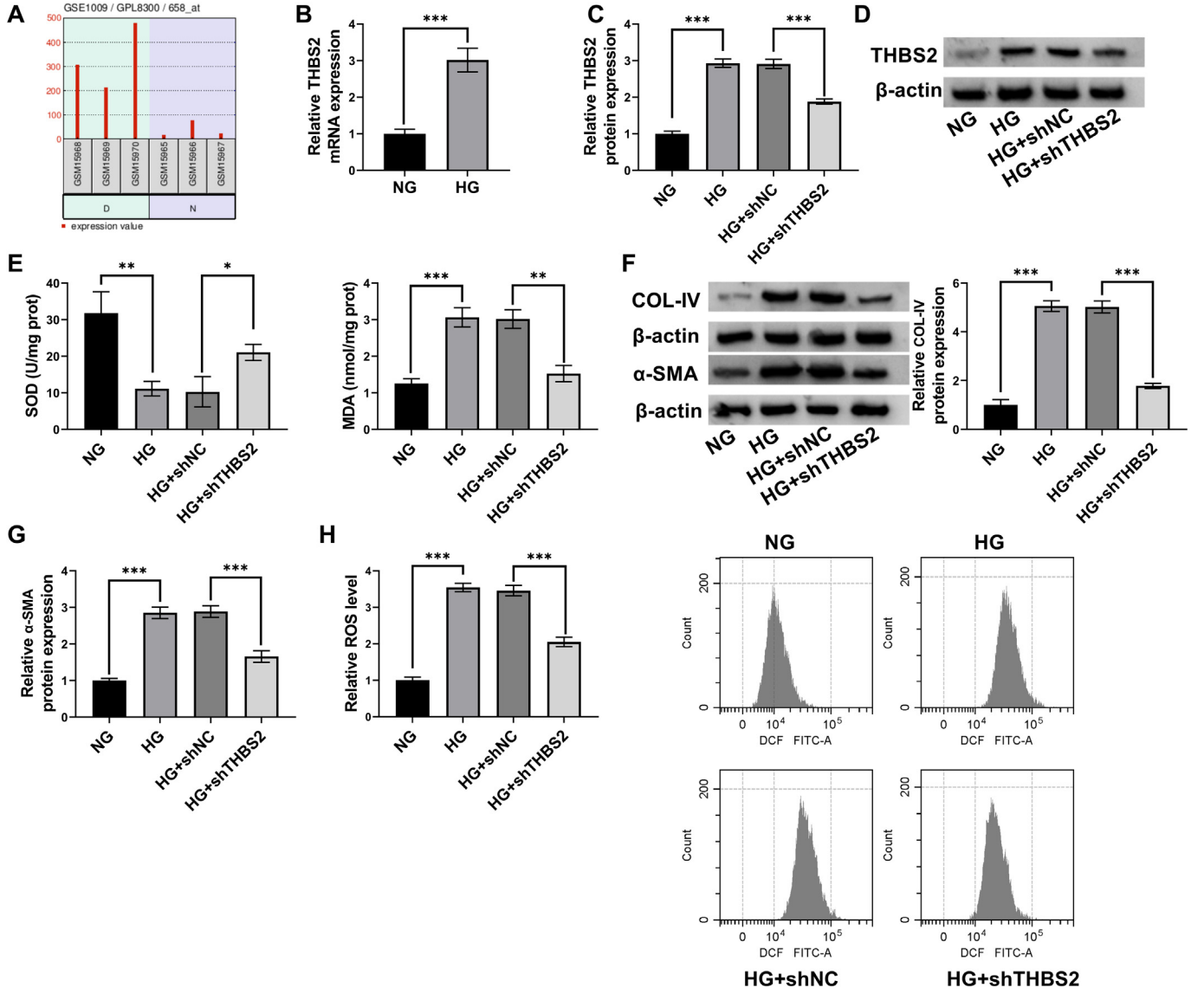

Fig. 5. Effect of shTHBS2 on oxidative stress and fibrosis in HG-induced mesangial cells. (A) GSE1009 database analyzed THBS2 expression in kidney glomeruli from 3 DN patients and 3 normal controls. (B) THBS2 mRNA expression was detected by qRT-PCR in mesangial cells treated with NG or HG (n = 3). (C–H) mesangial cells were divided into 4 groups, including NG, HG, HG + shNC and HG + shTHBS2 (n = 3). (C–D) THBS2 protein expression was examined using WB in each group. (E) SOD activity and MDA level were examined by the corresponding kits in each group. (F-G) COL-IV and α-SMA protein levels were tested using WB in each group. (H) ROS level was measured using DCFH-DA probe with flow cytometry in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

sion. Although Li et al. [12] established the association between THBS2 and DN at the multi-omics level, their analysis was based on global tissues and could not resolve cell-type-specific mechanisms. By using scRNA-seq, this study systematically revealed the direct role of THBS2 in driving oxidative stress and fibrosis in mesangial cells at single-cell resolution for the first time and carried out corresponding functional validation in vitro. This provides new experimental evidence for the cell-type-specific function of THBS2 and also provides a more refined rationale for the development of targeted intervention strategies against mesangial cells.

Of course, there are limitations. Our present work confirmed the functional phenotype of THBS2 and identified "oxidative phosphorylation" and "ribosome" pathways by GO/KEGG analysis, but the details of upstream regulation and downstream pathways (such as oxidative phosphorylation) of THBS2 have not been solved. As a next step, RNA-seq analysis of THBS2 knockdown mesangial cells will be performed to systematically screen the downstream target genes and signaling networks regulated by THBS2 knockdown mesangial cells. Besides, Co-IP and pathway rescue experiments are planned to verify the key interactions and functional pathways. Combined with the existing research basis, TGF-β/Smad, PI3K/AKT and other pathways may be involved in the regulation of THBS2, and its effect on ROS generation and the direct/indirect association with COL-IV and α-SMA also need to be further verified in the future. In terms of fibrosis phenotype, this study only examined COL-IV and α-SMA, which is inadequate. This study plans to use immunofluorescence technology in subsequent cell experiments to more comprehensively characterize the fibrosis of mesangial cells induced by HG. In addition, the expression and intracellular localization changes of key fibrosis components such as COL-IV, α-SMA and fibronectin (FN) were detected and observed at the same time, so as to verify the role of THBS2 in fibrosis more intuitively and multidimensively. Moreover, because the focus of our analysis was on identifying static HG-related differential targets, a differential gene analysis strategy was employed. However, this failed to reveal the dynamic pattern of THBS2 changes during the

disease progression. In the future, performing pseudotime or trajectory analyses to infer dynamic genetic changes during DN progression may provide us with further analytical perspectives.

It will be important to verify the localization of THBS2 in human tissues. However, due to the practical difficulties in obtaining paired and high-quality human DN renal tissue sections, clinical sample validation has not been carried out in this study. In the future, this study strives to supplement qRT-PCR/WB/immunohistochemistry (IHC) validation of THBS2 in clinical tissues to provide the most direct clinicopathological evidence. In addition, this study aimed to screen and validate the key genes associated with DN, but did not involve THBS2 inhibitor screening. After clarifying the pathogenic role of THBS2 in the future, our study plan is to carry out targeted studies: using PharmMapper and other databases to predict potential small molecule compounds, and verifying their function and efficacy through cell and animal models to promote their clinical transformation. The aim of this study is to evaluate whether systemic knockdown of THBS2 expression can ameliorate diabetic renal pathologies (e.g., urinary protein, glomerulosclerosis, collagen deposition, etc.) in a STZ-induced diabetic mouse model by tail vein injection of AAV-shRNA targeting THBS2. This *in vivo* validation is a future research focus for further mechanistic and translational studies.

In conclusion, our study identifies THBS2 as a novel mediator of oxidative stress and fibrosis in mesangial cells of DN via integrated scRNA-seq and multi-database analysis. These data suggest that targeting THBS2 may offer a promising therapeutic strategy for alleviating DN progression.

CRediT authorship contribution statement

Lin Lei: Writing – original draft, Validation, Resources, Project administration, Formal analysis, Conceptualization. **Jingxia Guo:** Project administration, Methodology, Formal analysis. **Xin Wang:** Supervision, Resources, Methodology, Investigation. **Min Liu:** Validation, Supervision, Software, Formal analysis, Data curation. **Chenxi Liu:** Software, Resources, Project administration, Formal analysis.

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Declaration of competing interest

The authors have no interests to disclose.

Supplementary material

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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